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TITLE

EFFICACY OF DBDMH (1,3-DIBROMO-5,5-DIMETHYLHYDANTOIN) AGAINST ERWINIA

TEST GUIDELINE

None

AUTHOR

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STUDY COMPLETION DATE

January 9, 2014

PERFORMING LABORATORY

Albemarle Corporation
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LABORATORY PROJECT ID

9504

PAGE COUNT

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C).

Submitter: Ann Oxford Date: 4.4.2014
Ann M. Oxford
Company: Albemarle Corporation

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GLP COMPLIANCE STATEMENT

This study does not meet the requirements of 40 CFR 160. Because the efficacy data is not being used to support human health claims, it is not necessary to conduct the testing under Good Laboratory Practices.

Submitter: Albemarle Corporation

Sponsor: Ann Oxford
Date 6·3·2014

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ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT

EFFICACY OF DBDMH (1,3-DIBROMO-5,5-DIMETHYLHYDANTOIN) AGAINST ERWINIA

I.	Protocol Number:	130830
II.	Sponsor:	Albemarle Corporation 451 Florida Street Baton Rouge, LA 70801
III.	Analytical Testing Facilities:	Albemarle Corporation Process Development Center Gulf States Road Baton Rouge, LA 70805
IV.	Test Start Date:	October 30, 2013
V.	Test Completion Date:	January 9, 2014
VI.	Test Article:	DBDMH (1,3-DIBROMO-5,5 DIMETHYLHYDANTOIN) Lots 090213 and 120112.
VII.	Objective:	The purpose of this study is to determine the efficacy of DBDMH against Erwinia.

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VIII. Overview:

The current project tested the efficacy of DBDMH on *Erwinia carotovorum* subsp. Carotovorum. Erwinia was chosen for its well-known ability to cause soft rot in wide variety of fruits and vegetables. Overall, the DBDMH treatment of tomatoes resulted in over 1 log reduction of the bacteria.

IX. Procedure:

Microorganisms and culture conditions

Erwinia carotovorum subsp. carotovorum (ATCC#15359) was purchased from American Type Culture Collection. The strain was maintained as a frozen glycerol stock and was sub-cultured in Luria-Bertani (LB) medium at 30°C prior to the experiments.

Inoculation

Overnight cultures of *Erwinia carotovorum* were pelleted, washed twice in PBS buffer, and titrated to a concentration of 10^8 CFU/ml. The tomatoes were inoculated with 5 log of *Erwinia* and air dried inside a biosafety cabinet for 1 hour prior to the treatment.

Biocide preparation and residual measurement

Each DBDMH lot (# 120112 and # 090213) was dissolved in tap water at 23°C to prepare a concentrate solution. The concentration of this solution was determined as total bromine by using N, N diethyl-p-phenylenediamine (DPD) colorimetric method and a Hach DR3400 spectrometer. The concentrate was then diluted to the test concentration with deionized water.

Treatment procedure

Each test tomato was sprayed within a biosafety cabinet by using a model 1550 Autojet Modular Spray system (Spraying Systems Co.). Two spray nozzles situated on each side of the test tomato will be used to deliver 100 ppm of the biocide for 45 seconds. This study tested two different DBDMH lots and at least 3 replicate tomatoes for each lot. Immediately after spraying, each tomato was drained of the excess biocide for one minute and transferred to a ziplock bag prefilled with 100 ml of 10% peptone solution. The rinse bag was rocked back and forth for 1 minute to dislodge the surviving bacteria from the sample.

Enumeration of Erwinia

One ml of the rinse solution was serially diluted in 10% peptone and plated onto LB agar for enumeration of the viable bacteria.

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X. Results

	Treated	Untreated	d_i
Ex.1	4.602	5.813	1.211
	4.477	5.954	1.477
	4.903	6.389	1.486
Ex.2	4.556	6.033	1.477
	4.301	6.255	1.954
	4.301	6.415	2.114
Mean	**4.523**	**6.143**	**1.620**
STDEV	**0.225**	**0.246**	**0.341**

Table 1. Concentration of *Erwinia* recovered from treated and untreated samples expressed in a log scale. Each of d_i values is the difference between untreated and treated samples (log reduction).
Statistical analysis: The mean log values of treated and untreated groups are significantly different (two-tailed T test, p=0.0001, α=0.05)

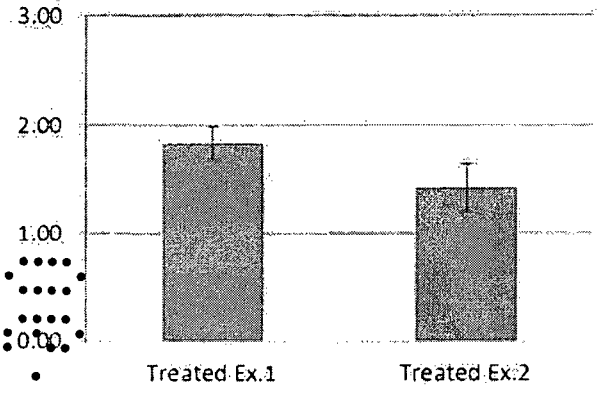

Figure 1. Each bar is a mean ± S.D. of log reduction of *Erwinia* in the treated samples.
Statistical analysis: One-tailed statistical T analysis with α=0.05 and t (d.f) =5 estimates a log reduction of 1.34 at a confidence interval of 95%.

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XI. Conclusion:

Approximately 100 ppm solution as bromine from DBDMH provided a statistically significant reduction of Erwinia on tomatoes.

XII. Project Approval:

Laura Gage
Laura Gage
Study Director

3/31/14
DATE